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CASE REPORT

Apoptosis of ileal crypt epithelia after allogeneic bone marrow transplantation without graft-versus-host disease

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Key Clinical Message

Intestinal crypt cell apoptosis may occur after allogeneic bone marrow transplantation without clinically overt graft-versus-host disease. We describe this phenomenon in a case of a 12-year-old girl who had segments of the ileum resected because of a relapse of acute lymphoblastic leukemia. The diagnostic difficulties are discussed.

Keywords

acute graft-versus-host disease, apoptosis, ileum, relapse of acute lymphoblastic leukemia.

Introduction

Apoptosis of crypt epithelia is the histological hallmark of low-grade acute intestinal graft-versus-host disease (aGvHD) after allogeneic bone marrow transplantation (BMT) [1]. The process results from immunological reactions which may lead to mucosal destruction. Since scant crypt epithelial apoptosis could also be induced by viral infection [2] or the toxic effects of medication [3], there is a search for a numerical threshold to separate those conditions from aGvHD [4]. However, the occurrence and degree of apoptosis after BMT without clinical disease is not well documented.

Patient Description and Methods

A 12-year-old girl suffered from T-acute lymphoblastic leukemia (ALL) with a t(11;19)(q23;p13.3) and MLL-ENL gene fusion. She received BMT due to recurring positive MRD after a reinduction course of first-line chemotherapy (German CoALL 08-09 Trial). The donor was her father (haploidentical phenotype). A conditioning regimen with non-TBI, fludarabine, ATG, and cyclophosphamide was initiated and she received an αβ-TCR/CD19-depleted graft. For GvHD prophylaxis, mycophenolate mofetil until day (d) +100 following BMT was used. She achieved a sustained neutrophil engraftment on d + 11 and maintained a complete donor chimerism at d + 15.

$CD56^+$ NK cells showed an early surge on d + 18 and $CD3^+$ cell counts of >200/μL were achieved on day +32. Despite rapid donor T-cell engraftment, no clinical signs of GvHD were detectable and no biopsies for GvHD diagnosis were taken. The patient was CMV IgG positive at the time of transplantation and received stem cells from a CMV IgG-positive donor. Prophylactic treatment with aciclovir was initiated and replaced by ganciclovir on d + 56 on account of reactivated CMV infection and consecutive marked unilateral retinitis. After d + 72 following BMT peripheral blood CMV-PCR results remained continuously detectable at low levels (9×10^{-3}cp/mL–2.2×10^{-2} cp/mL) and valganciclovir was continuously administered.

Five months after BMT, the girl developed repetitive intussusceptions as a result of an intestinal relapse of lymphoblastic leukemia. Four operations were performed, in

which a total of five segments of the ileum up to 12 cm in length were removed (d + 131 (one segment), d + 154 (one segment), d + 158 (two segments), d + 163 (one segment)). The postoperative courses were uneventful. Finally, the patient was discharged and received palliative chemotherapy.

Of those ileum segments, three were adequately fixed in 4% formalin solution, while the other two segments were unsuitable because of autolytic changes. Only one of the adequately fixed segments (removed d + 154) was removed prior to corticoid treatment. The routinely administered drugs from d + 140 to d + 154 were valganciclovir, cotrimoxazole, isocillin, omeprazol, folic acid and cefixime.

On gross inspection, the investigated segment revealed a bulky tumor (Fig. 1). The adjacent mucosa and the intestinal wall were unremarkable and showed no signs of inflammation or edema. The segment was consecutively cut in slices of about 3 mm. From each slice, one HE-slide was evaluated. Microscopical investigation revealed a relapse of the ALL with bulky disease and an extended leukemic infiltrate in the neighboring flat mucosa. In other areas, not infiltrated by the ALL, basal crypt epithelia undergoing apoptosis were increased. The apoptotic cells were clustered and amounted to eight per high power field (Fig. 2A). In contrast, most other parts of the mucosa gave no evidence of crypt epithelial apoptosis. Immunohistochemical staining for CMV and PCR tests for adenovirus were all negative. In regions which were infiltrated by the ALL only scant apoptosis was observed (Fig. 2B).

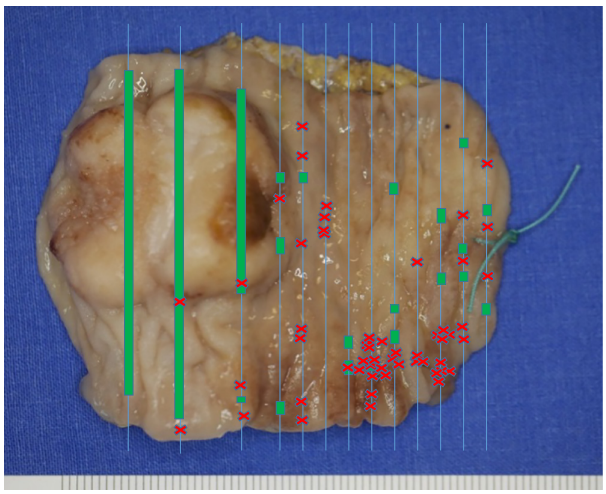

Figure 1. Segment of the ileum from the second (d + 154) operation revealing a bulky infiltrate of the ALL. The distribution of apoptosis, leukemic cells (extending beyond the borders of the grossly visible tumors, and the level of the slices are indicated. ▮, ALL Infiltrate; ✖, Apoptosis; |, Slice.

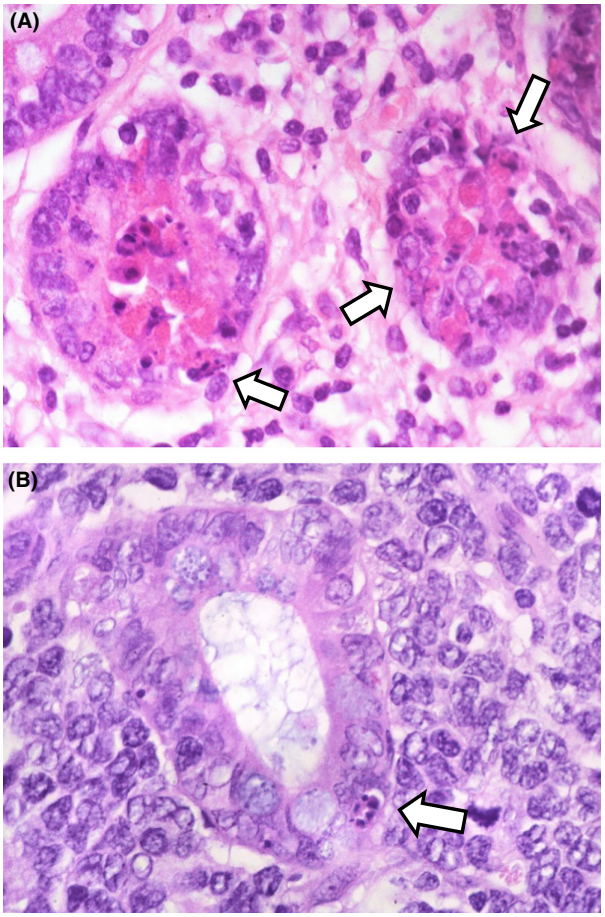

Figure 2. (A) Clustered apoptosis in an area of high apoptotic count, not affected by the leukemic infiltrate. No signs of enteritis in the lamina propria (this tissue block tested negatively for adenovirus by PCR analysis). (B) Single focus of apoptosis occurring in an area with leukemic infiltrate. The proliferating leukemic blasts occupied the lamina propria.

Discussion

After BMT, several mechanisms may cause apoptosis of the crypt epithelia, including toxic, infectious, and immunological as well as mechanical and ischemic processes. The patient was under treatment with several drugs, of which two are known to cause epithelial cell apoptosis. Proton pump inhibitors such as omeprazole have been shown to increase apoptosis in the gastric mucosa [5], but this medication may only result in a small increase and the apoptotic bodies did not cluster, which is in contrast to the observation in our case. Valganciclovir is a prodrug of ganciclovir, which can induce apoptosis in CMV-infected cells [6]. As the patient continued to have positive CMV-PCR the possibility of CMV enteritis has to be discussed. However, in the histology, there were no signs of

CMV enteritis and a PCR for CMV was negative in the tissue block with the highest count of apoptotic bodies. Thus, we may exclude the induction of apoptosis by this drug. To avoid the effect of corticosteroids on apoptosis [7], we only report the findings in a segment which was removed prior to corticoid treatment. Finally, there were no signs of enteritis or ischemic or mechanical injury in the removed ileal segment. Thus, we could attribute the increased number of apoptotic bodies to the immunological process of graft–versus-host interaction after BMT [1], which in this case affected only minor parts of the mucosa and therefore did not result in clinically evident aGvHD. Moreover, there were no clinical signs of GvHD in other organs. Since the induction of apoptosis results from the interaction of epithelia with allogeneic lymphocytes the dense leukemic infiltrate may have shielded the crypts from destruction. The relative lack of apoptosis in these areas presents a further argument against mechanical or ischemic injury, which would have affected neighboring mucosal regions more or less equally.

Irregularly distributed apoptosis has already been reported in intestinal aGvHD. Some authors claim that they are most prominent in the small intestine [8], as was found in our case. Thus, the ileum may be most vulnerable to damage in aGvHD. However, we were not able to compare our observation with other parts of the intestine.

In our case, we had the opportunity to map histologically a comparatively large area of intestinal mucosa. Only by systematic evaluation we were able to discover some regions of clustered apoptotic bodies in a vast area of only occasional apoptosis. This situation is completely different from the normal diagnostic situation, in which only minute particles of the mucosa removed by endoscopy come to histological evaluation. The opportunity to receive such a "hot spot" area is only marginal in this setting. Thus, our findings are not comparable to the papers which discuss the amount of crypt cell apoptosis needed for the diagnosis of acute intestinal GvHD. However, single apoptotic bodies found in intestinal mucosa after BMT were regarded by some to be insufficient for the diagnosis of GvHD [4], whereas others found even single apoptotic bodies alarming [9]. We conclude from our findings that single and even clustered apoptotic bodies of crypt epithelia may be an incidental finding after BMT, which become diagnostic for acute intestinal GvHD only in an appropriate clinical context. The need for a close correlation of histological and clinical findings cannot be overemphasized in such cases.

Conflict of Interest

The authors declare no conflict of interest.

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